

## Inhibition of *Salmonella* sp. Growth in Comparison with Antibiotics and Pineapple (*Ananas comosus*) Squeeze

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### Abstract

The purpose of this study to examine antibiotics inhibition growth of *Salmonella* sp. isolated from feces of broiler chickens to chloramphenicol, gentamicin, and tetracycline in comparison with pineapple squeeze. Swab sample from broiler chicken cloaca was cultured to nutrient broth media and incubated at 37°C temperature for 24 hours. Culture was compared with Mc Farland 3 standardization, and separated on surface of Muller Hinton Agar (MHA). Antibiotic sensitivity was tested by mean of chloramphenicol, gentamicin, and tetracycline in comparison with pineapple squeeze. The antibiotic disks (Oxoid) were put on the surface of MHA media which had inoculated with *Salmonella* sp. bacteria. Antibiotic sensitivity was determined based on formation of inhibition zone in surrounding of antibiotic disc. The result showed that *Salmonella* sp. bacteria growth was inhibited in  $\geq 21$  mm and  $\geq 18$  mm,  $\geq 7$  mm and  $\geq 9$  mm against chloramphenicol, gentamicin, tetracycline, and pineapple squeeze respectively. This research concluded that the *Salmonella* sp. cultured in this manner were sensitive to chloramphenicol and gentamicin. The inhibition growth of *Salmonella* sp. caused by chloramphenicol and gentamicin was significantly higher in comparison with pineapple (*Ananas comosus*) squeeze, meanwhile the *Salmonella* sp. bacteria was resistance against tetracycline. Our result suggest that both antibiotics chloramphenicol and gentamicin were recommended for antibiotic therapy in *Salmonella* sp. infection.

**Key words:** *Salmonella* sp., chloramphenicol, gentamicin, tetracycline, pineapple squeeze

### Introduction

Salmonellosis is among the most important broiler diseases. Salmonellosis caused economic problem in poultry husbandry such as labour cost, treatment cost, veterinary time, consultation fees, and cost of the antibiotics. Chemical therapy of bacteria coupled with improved management has been the important control strategy throughout the world. However, increasing problems of development of resistance in bacteria against antibiotics have led to the proposal of screening medicinal plants for their antibiotics activity. In many literature described that the plant are known to provide a rich source of botanical antibiotics. It is the purpose of this study to examine antibiotics susceptibility of *Salmonella* sp. isolated from feces of broiler chickens to chloramphenicol, gentamicin, and tetracycline in comparison with pineapple squeeze.

### Materials and Methods

The samples of feces were collected from 15 broiler chickens. The samples were sent for examination to the Microbiology Laboratory of Veterinary Faculty of Syiah Kuala University. The samples were cultured to nutrient broth media (merck. Oxoid Ltd. Basingstoke, Hampshire, England) and incubated at 37°C temperature for 24 hours.

### Bacteriological Procedures

Mac Conkey Agar (merck. Oxoid Ltd. Basingstoke, Hampshire, England) and Brilliant Green Agar are slightly selective and differential plating media mainly used for the detection and isolation of *Salmonella* sp. gram-negative organisms from feces sources. Cultures are incubated in humid air at 36°C for 48 hours. Cultures are examined each day for growth and any colonies are Gram stained and subcultured (i.e., transferred) to appropriate media as explained by Darmawi *et al.*, 2013).

### Gram Stain

In this study we obtain a clean glass slide to prepare a smear of each bacteria to be stained by taking a loopful of the bacteria (with a sterile loop) and spreading it over a small area in the center of the slide. The slide allowed the smear to air dry and then heat fix by passing the slide quickly through a flame. The slide placed on paper towels and add a drop or two of crystal violet to the smear to let set 1 minute. The slide washed gently the stain off with tap water carefully in order to being not to wash off bacteria. Then, Gram's iodine apply to let set 1 minute. To remove any excess stain or stain that has not adhered to the cell, the slide washed gently the iodine off with tap water and then add the decolorizing agent (95% EtOH) drop by drop until it runs clear. The decolorizing reagent washed off with tap water, and counterstain with safranin by adding 1-2 drops and let it set for 45 seconds. Finally, the slide rinsed with tap water, looked at under the microscope, and determined if bacterium is Gram-positive or Gram-negative. Gram-negative cells will pick up the counterstain and appear red or pink as described by previously study (Health Protection Agency, 2007; Darmawi *et al.*, 2011; Darmawi *et al.*, 2013) with certain modification.

### Biochemical Assayed

All isolates were identified according to the methods advocated by Edwards and Ewing (1962). The specific methods involved were colonial characteristics on media including size, inability to swarm, ability or inability to ferment lactose. Specific tests such as carbohydrate namely: Mannitol, Sucrose, Lactose, Glucose, Triple Sugar Iron Agar, indole formation, Methyl Red (Oxoid), Voges Proskauer (Oxoid), and Sulfic Indole Motility, Simmon's Citrate (Oxoid) tests were done as described by Darmawi *et al.*, 2011; Darmawi *et al.*, 2013) with certain modification.

### Preperation of Pineapple Squeeze

Pineapple (*A. comosus*) were procured from local market (Banda Aceh, Indonesia). The crude aqueous squeeze of the pineapple fruits were prepared by pineapple squeezer.

### Kirby-Bauer Antibiotic Testing

Cultures were compared with Mc Farland 3 standardization, and separated on surface of Muller Hinton Agar (MHA). Organisms are also tested for antibiotic susceptibility by the Kirby Bauer method. Antibiotic sensitivity was tested by mean of chloramphenicol and gentamycin. The antibiotic discs (Oxoid) and pineapple extract were put on the surface of MHA media which had inoculated with *Salmonella* sp. as described by Darmawi *et al.*, 2011; Darmawi *et al.*, 2013) with certain modification. The blank antibiotic disks (Oxoid) were put on the center, and the flooded pineapple squeeze and antibiotic discs were put in the surface of MHA media which had inoculated with bacteria. After overnight incubation, the zone of inhibition is appear on the surrounding of disk.

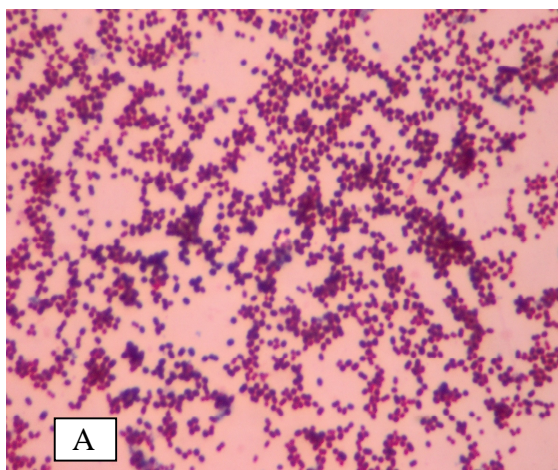
### Results and Discussion

The samples were positive for species of the *Salmonella* sp. bacteria isolated from feces of broiler chickens as shown in Table 1.

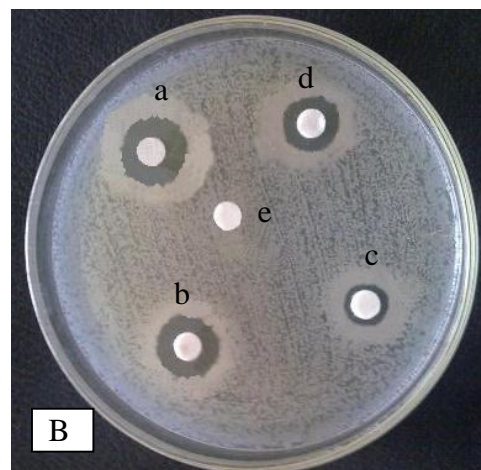
Table 1. *Salmonella* sp. bacteria isolated from feces of broiler chickens

Test	Biochemical Assayed									
	Mannitol	Sucrose	Lactose	Glucose	SIM	TSIA	Simmon cytrate	MR	VP	Indol
Reaction	+	-	-	+	+	+	+	+	-	+

Culture separated on surface of Mac Conkey or Brilliant Green Agar develop bacteria colony. Under microscope, Gram-negative cells appeared red or pink colour as shown in Figure 1A. The zone formed in this manner is inhibit *Salmonella* sp. growth shown in Figure 1B.



**Figure 1A.** Gram negative bacteria (red or pink colour)



**Figure 1B.** Inhibition of *Salmonella* sp. growth caused by antibiotics and pineapple squeeze. a = chloramphenicol, b = gentamisin, c = tetrasiklin, d = pineapple squeeze, e = blank disc

Diagnostic laboratory test in which microorganisms from an broiler feces are grown in the laboratory on media MHA to identify antibiotics that will be effective in destroying the organism (Goldstein *et al.*, 1984). The result showed that choramphenicol and gentamycin inhibit *Salmonella* sp. growth. Although tetrasiklin and pineapple extract was also able to formed inhibition zone, but there were not including sensitive as shown in Table 2.

Table 2. Diameter inhibition zone of antibiotics and pineapple squeeze

Antibiotic	Zone (mm)
Chloramphenicol	≥ 21
Gentamycin	≥ 18
Tetracycline	≥ 7
Pineapple squeeze	≥ 9
Blank disc	0

Antibiotics are usually given in an effort to eliminate the spread of pathogen bacteria. Therapy with antibiotics is usually recommended for a case to eliminate the carrier state and control secondary or associated infection. Although antibiotics have been reported to have little or no effect on the clinical course of the salmonellosis. Depending on the severity and the type of bacteria, the antibiotic may be given. If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the antibiotics disk where bacteria are not capable of growing called a zone of inhibition (Goldstein *et al.*, 1984; Andrews, 2001; Turnidge *et al.*, 2003) as described by Darmawi *et al.* (2011).

Sometimes, multiple antibiotics are used to decrease the risk of resistance and increase efficacy. Antibiotics only work for bacteria and do not affect viruses. Antibiotics work by slowing down the multiplication of bacteria or killing the bacteria. The most common classes of antibiotics used in medicine include penicillin, cephalosporins, aminoglycosides, macrolides, quinolones and tetracyclines. Do not take antibiotics for longer than needed. Long term use of antibiotics leads to resistance and chances of developing opportunistic infections.

## Conclusions

Inhibition growth of *Salmonella* sp. caused by chloramphenicol and gentamicin was significantly higher in comparison with pineapple (*Ananas comosus*) squeeze, meanwhile the *Salmonella* sp. bacteria was resistance against tetracycline.

## Acknowledgements

We wish to thank the chairman and all theaching staff of Microbiology Laborarory, Faculty of Veterinary Medicine of Syiah Kuala University that frendly pleased us to conduct this research.

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